Sayre

Studies of Methods of Determining Total Organic Nitrogen in Sewage

STUDIES OF METHODS OF DETERMINING TOTAL ORGANIC NITROGEN IN SEWAGE

BY

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I. INTRODUCTION

For several years, the determination of total organic nitrogen has been considered of great importance by the agricultural, physiological, and water chemists. The extensive use of the Kjeldahl method, the only method applicable to such materials as sewage and blood and the one most convenient of application to most other materials, has brought out many real or supposed sources of error in the method. The attention of many investigators has been given to a study of the causes of the irregularities in the method and to an attempt to devise modifications of the method by means of which these irregularities might be avoided.

II. THEORETICAL

In theory, the Kjeldahl method for the determination of nitrogen in organic substances is very simple. Boiling sulfuric acid is used to decompose the organic compounds and to unite with the nitrogen to form ammonium sulfate. The nitrogen of inorganic compounds such as that of sodium nitrate is driven off as nitric acid and is not included in the result unless a special modification of the method is made to convert this in-

organic nitrogen into ammonium sulfate. If nitrogen is present as free ammonia as it is in sewage - or as an ammonium salt, the sulfuric acid converts it immediately into ammonium sulfate. At the end of the digestion, the liquid is diluted with nitrogen-free water, enough strong sodium hydroxide solution is added to make the solution alkaline, and the liberated ammonia is distilled off. The ammonia in the distillate is determined either by titration with a standard acid or by nesslerizing and comparing the color produced with that of standards containing known quantities of ammonium chloride.

The method is often modified by the addition to the acid mixture of some substance to raise the boiling point during digestion or of some substance to act as a catalyst. No attempt will be made to describe the various modifications of the method for there are almost as many modifications of the method as there are laboratories using it.

A SOURCES OF ERROR

Needless to say, the errors are always toward low results. The one source of error inherent in the Kjeldahl method which has received most attention is the failure of the sulfuric acid digestion mixture to convert all of the nitrogen of organic compounds into ammonium sulfate and to hold all of the ammonia in combination until the end of the digestion. This may be due to one or more of the following causes: first, incomplete decomposition of the organic nitrogenous

compounds; second, loss of nitrogen due to the presence of other reacting substances, such as nitrates, nitrites, and chlorides; third, loss of nitrogen by volatilization of ammonia from the acid mixture. Besides these, there may be loss of nitrogen in distillation. These possible sources of error will be discussed in the order given.

1. Incomplete digestion.

The earlier outlines of the method directed that the digestion with sulfuric acid be continued until the digestate assumed a pale straw color or became colorless. Phelps[9] has shown that nothing short of complete loss of color is sufficient for sewage. He obtained distinctly higher results for total organic nitrogen when he digested samples to complete loss of color than when he digested the same samples to a pale straw color. Glimm[3], speaking of the Kjeldahl method as applied to substances other than sewage, states that complete loss of color does not always show complete conversion of the organic nitrogen into ammonium sulfate. Many organic nitrogen compounds dissolve in hot, concentrated sulfuric acid to give colorless solutions which still retain quantities of nitrogen in the original organic combination. Glimm recommends digestion for ten or more minutes after complete loss of color. In the more recent literature on the subject, it is generally directed that the digestion be continued at least thirty minutes after complete loss of color.

Nolte[7], states that the addition of sugar during

digestion is necessary in the determination of nitrogen in diphenylamine, methylindole, and tetramethylammonium bromide. In some other cases, sugar has no effect, and may even cause loss of nitrogen in analyses of acetanilide, uric acid, and caffeine. The complex nature of sewage suggests at once the bearing of these facts upon the determination of nitrogen in sewage and indicates the possibilities of error.

2. The effect of nitrates and nitrites.

Many have reported loss of nitrogen due to the presence of nitrates and nitrites. Compounds of nitric acid, in the presence of organic matter and sulfuric acid, may be reduced to nitrous acid. Nitrous acid may react with ammonia or with amino nitrogen to give free nitrogen. Von Spindler[14] claims that nitrates cause loss of nitrogen from urea because of the action of nitrous acid. Segawa[12] shows that nitrates cause loss of nitrogen from calcium cyanimide but that the loss can be prevented by reducing the nitrate content below 9.1 per cent. He attributes the loss to a reaction between cyanimide and nitrous acid from the reduction of nitrates. Schenke[11] maintains that large quantities of nitrites cause low results.

In an investigation carried out under the direction of Penny[8], Referee of the Association of Official Agricultural Chemists, on standardized nitric acid, most of the analyses showed low results for nitrogen determined by the Kjeldahl

method. When cane sugar and organic nitrogenous substances such as fish scrap were added, the results were still lower.

3. Volatilization of ammonia.

If the digestion is continued until there is a considerable loss of sulfuric acid, the loss of nitrogen in the form of ammonia may be important. Self[13] and Hibbard[14] mention loss of nitrogen when the temperature of the acid mixture rises above a certain point. When potassium sulfate is used, the loss increases as the liquid approaches the composition of acid potassium sulfate. In a very concentrated liquid, almost all of the ammonia may be volatilized in a very short time.

4. Errors in distillation.

Errors of some magnitude enter into the operation of distilling. Bardach[1] states that a considerable error is often due to the fact that the ammonia is not completely expelled from the flask, and to incomplete absorbtion in the acid receiver. He recommends testing the steam with litmus to show complete removal of the ammonia and the use of two receivers to assure complete absorption.

In contrast with the last statement, is the opinion of Klinkerfues[5] who advises the use of an amount of sulfuric acid in the receiver insufficient to neutralize the ammonia. The titration is then completed with the same acid. The advantages of using only one reagent in the titration are obvious.

Benedict[2] claims that the ammonia is soon expelled from the flask but adheres to the cold condenser tube. He draws off the condenser water near the end of the distillation and allows the tube to become hot in order to expell all of the ammonia.

An error of another kind is mentioned by Kober[6]. He gives the equation:

$$MgCl_2 + 2\ NH_4OH = Mg(OH)_2 + 2\ NH_4Cl.$$

He claims that the presence of magnesium or calcium salts causes the ammonia to distill slowly and often leads to variable results.

B THE WORK ON SEWAGE BY PHELPS[9].

The foregoing will be sufficient to indicate the irregularities of the Kjeldahl method as applied to various substances and will show the trend of the investigations of the method. The work of Phelps is of particular interest from the standpoint of sewage analysis. An account of his work will summarize, in part, the foregoing and will confine the discussion directly to the application of the method to sewage analysis.

Phelps had employed the simplified Kjeldahl method to sewages with uniformly good results. On sewage effluents, particularly on the better grade of effluents, however, he had obtained suspiciously low results. These did not appear regularly, and when they did occur, the duplicate analysis

never agreed. At about the same time, similar difficulties were reported from other laboratories. Phelps made an experimental study of the Kjeldahl method and its modifications in order to explain the occasional failure of the simplified method.

The possibilities of error due to the following causes were investigated: first, mechanical loss during digestion; second, the presence of chlorides, nitrates, and nitrites; third, imcomplete digestion; and fourth, the failure of the digestion to oxidize certain organic substances whose decomposition could be quickly completed by the use of potassium permanganate at the end of the digestion.

Phelps reported that the mechanical loss during digestion is roughly one per cent of the total and is, therefore, negligible. He thinks that there is hardly any question but that chlorides, nitrates and nitrites react with the nitrogenous matter of sewage in such a way as to cause loss of nitrogen. In his experimental studies, however, he found no measurable loss of nitrogen from samples which contained 6 to 10 parts per million of total organic nitrogen and as much as 3600 parts per million of chlorine. He added various amounts up to 10 parts per million of nitrogen as nitrate and the same as nitrite and 10 parts per million of nitrate with 5 parts per million of nitrite and vice versa to a sewage containing 5.5 parts per million total organic nitrogen, and found no loss of nitrogen due to their presence. He concludes,

therefore, that chlorides per se cause no loss of nitrogen and that nitrites and nitrates in small quantities cause no serious loss. As has already been stated, he found that completeness of digestion is of great importance. He thinks that most of the difficulties encountered are due to incomplete digestion. In some cases, he found that the use of permanganate had no effect, while, in other cases the effect was considerable. He, therefore, advises its use in all cases.

The procedure recommended by Phelps is the digestion of 10 cc. of sample with 0.1 gram of copper sulfate and 5 cc. concentrated sulfuric acid until complete loss of color; the addition of small crystals of permanganate until a color persists; dilution of the digestate to 500 cc.; steam distillation of 100 cc. or more of this solution; and determination of ammonia by nesslerizing 50 cc. of the distillate. He recommends this method for simplicity and claims that it is reliable for various types of sewages and sewage effluents. He claims that the method is accurate to 0.5 parts per million on sewages containing 20 parts per million or less of total organic nitrogen.

III EXPERIMENTAL

A Decomposition of sewage on standing.

A study was made of the change of organic nitrogen into ammonia occurring when sewage stands for some time in bottles. A comparative study was also made of the results obtained by digesting sewage without removing the ammonia nitrogen with those obtained by distilling off the ammonia and determining the total organic nitrogen in the residue. The method of treatment of each sample was as follows: Upon the first sample, four determinations were made, namely ammonia nitrogen by direct nesslerization, ammonia nitrogen by distillation, total organic nitrogen in the residue after distillation, and total organic nitrogen plus ammonia nitrogen in the same operation. Then the sample was divided into two portions. One portion was kept in a stoppered bottle at room temperature. To the other portion, about 10 cc. of chloroform was added and the sample placed in a refrigerator. After an interval of two or more days, the four determinations mentioned above were repeated on each portion. The results are shown in Table I.

Table 1.- The decomposition of sewage on standing and a comparison of the results obtained by determining total organic nitrogen with and without first removing the ammonia nitrogen.

Parts per million.

Days standing	Chloroform	Ammonia nitrogen by distillation	Total organic nitrogen in residue	Sum of these	Total organic nitrogen plus ammonia determined together	Ammonia nitrogen by direct nesslerization
			SAMPLE 1			
0		56	33	89	83	32
2	without	50	20	70	60	50
2	with	46	22	68	68	42
			SAMPLE 2			
0		48	46	94	92	50
2	without	(64)	20	84	92	66
2	with	38	38	76	88	(54)
7	without	96	20	116	112	86
7	with	66	38	104	92	80

Not much accuracy can be claimed for the results. They show that organic nitrogen is changed into ammonia nitrogen as the sewage undergoes decomposition and that this change is slower when the sample is chloroformed and kept cold. It will be observed that the sum of the ammonia nitrogen by distillation and of the total organic nitrogen in the residue usually approximates the sum of the two when determined in the same operation. The fact that the two values check no more closely may well be due to unfamiliarity with the method. This point will be taken up again and the results shown in Table 7.

B Accuracy of the ammonia data.

The accuracy of the various determinations to be employed in the study was investigated. Forty direct nesslerizations were made for the purpose of determining:

(1) How closely duplicate determinations could be made to check each other.

(2) Whether the time of settling of the coagulant, the clearness of the supernatant liquid, and the final readings were dependent upon the amounts of copper sulfate and of sodium hydroxide added, and, if so, in what manner.

The forty nesslerizations were made on three samples on different days. In each case, the sewage was diluted with about an equal volume of water. This diluted sewage was allowed to stand for a few minutes and then the upper liquid was siphoned off and used in order to have samples free from much

solid matter and at the same time low enough in ammonia nitrogen content to fall within the range of accurate reading. Samples 1 and 3 were fresh sewage. Sample 2 was the same as sample 1 except that it had been chloroformed and kept in an ice box for two days. The results are shown in Table 2.

Table 2.-The accuracy of direct nesslerization and the effect of different amounts of coagulants added to fifty cubic centimeters of diluted sample.

Sample 1.

No.	10% copper sulfate added	33% sodium hydroxide added.	Ammonia nitrogen (Parts per million)	Remarks
1.	.3 cc.	.3 cc.	23	
2.	.3 "	.5 "	20	
3.	.3 "	.8 "	20	
4.	.3 "	1.2 "	23	
5.	.5 "	.3 "	23	
6.	.5 "	.5 "	..	
7.	.5 "	.8 "	23	
8.	.5 "	1.2 "	(26)	Final tube cloudy
9.	.8 "	.3"	19	
10.	.8 "	.5 "	19	
11.	.8 "	.8 "	19	
12.	.8 "	1.2 "	19	
13.	1.2 "	.3 "	23	
14.	1.2	.5	22	" " "
15.	1.2	.8	22	
16.	1.2	1.2	21	" " "

Sample 2.

1.	.3 cc	.5 cc.	22	
2.	.3 "	.8 "	21	
3.	.3 "	1.2 "	22	Final tube cloudy
4.	.5 "	.5 "	22	" " "
5.	.5 "	.8 "	23	" " "
6.	.5 "	1.2 "	..	Too cloudy to read.
7.	.8 "	.5 "	23	Final tube cloudy
8.	.8 "	.8 "	22	" " "
9.	.8 "	1.2 "	21	" " "
10.	1.2 "	.5 "	..	Too cloudy to read
11.	1.2 "	.8 "	22	
12.	1.2 "	1.2 "	..	" " "

Sample 3.

1.	.3 cc	.4 cc	21	
2.	.3 "	.8 "	21	
3.	.3 "	1.2 "	20	
4.	.5 "	.4 "	20	
5.	.5 "	.8 "	20	
6.	.5 "	1.2 "	19	
7.	.8 "	.4 "	..	Final tube cloudy,
8.	.8 "	.8 "	19	
9.	.8 "	1.2 "	20	
10.	1.2 "	.4 "	..	Too cloudy to read
11.	1.2 "	.8 "	19	
12.	1.2 "	1.2"	19	

The length of time between the addition of the coagulants and taking out the portions for nesslerization was approximately 1 1/2 hours in sample 1, 3/4 hour in sample 2 and 2 1/4 hours in sample 3.

In all of these determinations, the portion removed from the supernatant liquid and diluted for nesslerization was 1 cc. An error of one drop (about .05 cc.) would introduce an error of one part per million into the result. If we consider, also, that every final nessler tube contains some of the coagulants and many of them contain small amounts of suspended matter, both of which cause cloudiness, an accuracy of one part per million is the best that can be expected. The table shows that the difference between the highest and lowest results was usually 2 parts per million and that this difference did not exceed 4 parts per million except when very cloudy tubes were obtained.

The table does not show that any particular combination of copper sulfate and sodium hydroxide is to be preferred. There was some reason for thinking that large amounts of the coagulants often caused cloudy tubes. The proper amounts to use probably depend somewhat on the amounts of clay and of other solids in the sewage.

Determination of ammonia nitrogen by distillation is not as rapid as by direct nesslerization and, according to Phelps , is less accurate than by direct nesslerization because of the well known fact that some substances, such as

urea, egg albumin, and gelatin, are partly decomposed by hot alkalies and are converted into ammonia during distillation.

The effect of adding .5 cc. of strong sodium hydroxide solution to 100 cc. of a solution containing 50 parts per million of nitrogen as urea, distilling off 50 cc., and determining the ammonia nitrogen in the distillate is shown in Table 3.

Table 3.-The decomposition of urea by a hot alkali.

(Parts per million)

No.	Organic nitrogen present	Nitrogen as ammonia in distillate.
1.	50	3.8
2.	50	2.8
3.	50	3.5

The results, 3.8, 2.8, 3.5 parts per million check each other within the limits of accuracy of the method or nearly so. Since only partial decomposition of the urea was to be expected here, whether these results check each other or not is unimportant. They serve to show that some organic nitrogen compounds found in sewage do yield ammonia during distillation. This accords with the statements of Phelps and of others and leads one to consider the determination of ammonia nitrogen by direct nesslerization the more accurate as well as the simpler method.

On the other hand, distillation offers less trouble with cloudy tubes. In three determinations on each of two samples, the results checked with each other as closely as did those from direct nesslerizations. The results of these determinations together with the results of direct nesslerizations on the same samples are shown in Table 4.

Table 4.-Comparison of ammonia nitrogen determinations by direct nesslerization and by distillation.

(Parts per million)

	Sample 1.		Sample 2.	
No.	By distillation	By direct nesslerization.	By distillation.	By direct nesslerization.
1.	11.0	9.0	21.3	cloudy
2.	9.6	9.5	20.7	cloudy
3.	9.8	...	20.9	22
Average	10.1	9.25	20.9	22

C.-Accuracy of the total organic nitrogen data.

Theoretically, the titration of the distillate with N/50 acid when the ammonia nitrogen in it amounts to more than about ten parts per million should be more accurate than nesslerization of an aliquot part. This can be shown as follows: In all cases the sample digested is taken as 200 cc.

1 cc. N/50 H_2SO_4 = 1 cc N/50 nitrogen $= \frac{14}{50 \times 1000}$ = .00028 gr. nitrogen. .00028 gr. in 200 cc. = .0014 gr. = 1.4 mg. in one liter.

In titrating with congo red as indicator, the end point can probably be consistently read within a range of ± .3 cc. of acid. This gives a variation of ± .42 (1.4 x .3) parts per million in the result. This variation is obviously the same on high total organic nitrogen determinations as on low ones.

If 200 cc. is digested, the digestate diluted and 200 cc. of distillate collected, the aliquot part of this last 200 cc. which can be used for nesslerization will depend upon the quantity of nitrogen present. If the total ammonia nitrogen in the distillate is 50 parts per million, not more than .5 cc. can be nesslerized without making the nessler reading so high as to be inaccurate. This gives 2000 as a factor. If the correct reading is 2.5, the result is .025 x 2000 = 50 parts per million. One cannot expect to read the nessler tubes more accurately than to .1. A reading of 2.6 gives .026 x 2000= 52 parts per million. In other words, variations of ± 2 parts per million are to be expected on samples containing 50 parts per million. In a sewage containing 10 parts per million, 2.5 cc. for nesslerization, gives the same reading as .5 cc. of the stronger sewage. This gives a factor of 400. If the correct reading is 2.5, the result is .025 x 400 = 10.0 parts per million. If in this case the observer makes an error of +.1 in the reading the result is .026 x 400 = 10.4 parts per million. From these calculations, it appears that greater accuracy should be given by nesslerization only when the ammonia content of the distillate corresponds to 10 parts per

million or less in the sample.

A practical comparison of the two methods gave results more favorable to the titration method than was predicted by the calculations. The results of the comparisons are shown in Tables 5 and 6.

Table. 5.-Total organic nitrogen plus ammonia nitrogen in raw sewage by nesslerizing the distillate.

(Parts per million)

No.	Sample 1.	Sample 2.
1.	54.	32
2.	48	44
3.	51	44
4.	54	36
5.	(76)	44

From both the theoretical and practical considerations, it was evident that nesslerization of the distillate was not accurate enough for the purpose of the investigation. Accordingly, titration was employed in all of the later determinations. Usually the ammonia was distilled into an excess of N/50 sulfuric acid and the excess acid titrated with N/20 sodium hydroxide. Later it was found that the work was simpler and more accurate when the amount of acid in the receiver was not sufficient to neutralize all the ammonia as suggested by Klinkerfues[5]. The titration was then completed with the same acid. (See Table 6.)

The results of these determinations in chronological order show better checks obtained toward the latter part of the work, largely due to the elimination of sodium hydroxide in the titration and to the use of larger samples thus reducing the factor.

Table 6.-Total organic nitrogen plus ammonia nitrogen in various substances by titrating the distillate.
(Parts per million)

Substance	Vol. used	Results of duplicate determinations					Variation
Raw sewage	25 cc.	54.8	56.	49.3	51.7		± 1.2
Raw sewage	100 cc.	48.4	51.8	47.6	44.8	44.8	+± 1.8
Raw sewage	100 cc.		31.2	36.2	33.0		± 5.0
Raw sewage	100 cc.	47.8	43.5	41.4	46.4		± 3.2
Solution of urea	100 cc.	55.5	50.0	50.0	55.5	50.0	± 2.7
" " "	200 cc.	48.9	48.2	49.2	49.6	48.6	± .7
Solution of beef broth	200 cc.	10.7	10.2	11.1	10.9	10.2	± .45
Raw sewage	200 cc.	38.5	38.4	38.3	38.1		± .2
Raw sewage	200 cc.	47.8	47.1	47.8			± .35
Raw sewage	200 cc.	37.2	36.4	37.3			± .45
Raw sewage	200 cc.	15.8	14.8				± .5
Sewage effluent	200 cc.	16.4	14.8	15.2			± .8
" " "	200 cc.	14.5	14.8	14.7			± .15
Raw sewage	200 cc.	27.5	27.8	26.0			± .9

Raw sewage	200 cc.	35.6	34.0	34.7	36.1			± 1.05
Solution of urea	200 cc.	50.3	49.7	50.1	51.2	51.2	50.8	± .3
" " " "	200 cc.	51.6	51.2	50.5	50.5	50.8		± .55
Raw sewage	200 cc.	36.3	36.3	35.6				± .35
Raw sewage	200 cc.	29.8	29.5	(26.5)				
Raw sewage	200 cc.	38.6	38.5	37.8				± .4
Raw sewage	200 cc.	47.5	46.1	47.7				± .8
Raw sewage	200 cc.	36.4	37.5	38.1				± .85
Raw sewage	200 cc.	53.7	54.6					± .45
Sewage effluent	200 cc.	13.9	13.0					± .45

The variation is found by dividing the difference between the highest and the lowest result by 2.

The results show that an accuracy of ± 1.05 parts per million or better was obtained in all of the determinations except the first five and that an accuracy of ± .5 parts per million was usually obtained. These are much better checks than were obtained by nesslerizing an aliquot part of the distillate.

D.-Comparison of the results obtained by determining total organic nitrogen with and without first removing the ammonia nitrogen.

Four sets of determinations were made in an effort to learn whether there is any loss of nitrogen when the sewage is digested without first distilling off the ammonia nitrogen. The results are shown in Table 7.

Table 7.-Total organic nitrogen determined with and without first removing the ammonia nitrogen.

	Sample 1.	Sample 2.	Sample 3.	Sample 4.
Ammonia nitrogen by distillation.	9.6 9.7	21.3 20.7 20.9	28.7 26.6	14. 11.1
Average	9.6	21.0	27.65	21.55
Total organic nitrogen in the residue	6.2 6.4	29.1 28.2 28.3	27.7 27.2	2.9 2.2
Average	6.3	28.5	27.45	2.55
Sum of averages	15.95	49.5	55.10	15.1
Total organic nitrogen plus ammonia nitrogen determined together	15.8 14.8	47.8 47.1 47.8	53.7 54.6	13.9 13.0
Average	15.3	47.5	54.15	13.45

It seems significant that the sum of the total organic nitrogen and ammonia nitrogen was in every case higher when they were determined separately than when determined in one operation. The difference is so small, however, as to leave unwarranted the conclusion that nitrogen is lost when the sample is digested without first removing the ammonia nitrogen.

E.-The effect of nitrates and nitrites.

The effect of nitrates and of nitrites during digestion was investigated by adding a known amount of sodium nitrate or of sodium nitrite to the sample immediately before

digestion. The results are shown in Tables 8 and 9.

Table 8.-The effect of the addition of nitrate nitrogen immediately before digestion upon total organic nitrogen.

(Parts per million)

Sample 1.
Total organic nitrogen

Substance	A	B	C	D	Average
Sewage	27.9	26.6	(33.7)		27.25
Sewage + 5 ppm nitrate nitrogen	25.6	26.3	24.5		23.5
Sewage + 10 ppm nitrate nitrogen	26.5	24.6	23.3		24.8
Sample 2.					
Sewage	27.5	27.8	26.0		27.1
Sewage + 5 ppm nitrate nitrogen	24.6	24.3	24.5		24.5
Sewage + 10 ppm nitrate nitrogen	26.0	24.6	23.5		24.7
Sample 3.					
Sewage	36.3	36.3	35.6		36.1
Sewage + 10 ppm nitrate nitrogen	30.2	30.9	31.3	30.6	30.7

Sample 4.				
Sewage	47.5	46.1	47.7	47.1
Sewage + 2.5 ppm nitrate nitrogen	48.4	44.8		46.6
Sewage + 5.0 ppm nitrate nitrogen	44.1	44.7		44.4
Sewage + 10.0 ppm nitrate nitrogen	42.5	42.7		42.6
Sample 5.				
Sewage	36.4	37.5	38.1	37.3
Sewage + 5.0 ppm nitrate nitrogen	36.2	35.9		36.05
Sewage + 10.0 ppm nitrate nitrogen	33.8	34.9		34.3
Sewage + 15.0 ppm nitrate nitrogen	32.5	33.4		33.0

From the first two samples, there seems to have been some loss of nitrogen due to the presence of the nitrate. From the other three samples, the loss was considerable and increased with the increase of nitrate. According to the statements of Von Spindler[14], Segawa[12], Schenke[11], and of the fertilizer chemists in general this loss was to be expected. On the other hand, the results do not agree with the results obtained by Phelps[9]. The same may be said of the results shown in Table 9.

Table 9.-The effect of the addition of nitrite nitrogen immediately before digestion.

Sample 1.

Total organic nitrogen.

Substance	A	B	C	Average
Sewage	29.8	29.5	(26.5)	29.6
Sewage + 2.5 ppm nitrite nitrogen	28.0	28.0	....	28.0
Sewage + 5.0 ppm nitrite nitrogen	26.2	28.0	....	27.1
Sewage + 10.0 ppm nitrite nitrogen	26.2	25.4	....	26.6
Sample 2.				
Sewage	38.6	38.5	37.8	38.3
Sewage + 2.5 ppm nitrite nitrogen	38.9	38.2	....	38.5
Sewage + 50 ppm nitrite nitrogen	37.5	37.1	....	37.3
Sewage + 10.0 ppm nitrite nitrogen	36.7	36.0	....	36.3

These two sets of determinations in the presence of nitrites were made in exactly the same manner except that the flasks were placed on the digestion rack and on the stills in the reverse order for the purpose of checking up possible discrepancies due to leaks in the stills and connections and to the fact that some burners gave higher temperatures than others. The results seem to indicate a small but distinct loss of nitrogen when nitrites are present and that this loss increases with an increase in the amount of nitrite present.

In reading this and all other tables, the fact should be kept in mind that all the observed losses are not far beyond the limits of accuracy of the method.

F.-Blank determinations on stills.

Some blank determinations were made on dirty stills to learn whether it was advisable to steam out the stills before using. About 350 cc. of nitrogen-free water was put into a flask, 200 cc. distilled over, and 50 cc. of the distillate nesslerized. Two of the stills had been recently used; the other had not. The quantities of nitrogen removed by the 200 cc. distillates were,respectively, .044 mg, .04 mg., and .024 mg. Since 200 cc. samples were ordinarily used for determinations, these quantities times the factor, 5, give the possible errors in parts per million, manely .22 part, .20 part, and .12 part per million.

In view of these results, it hardly seems necessary to steam the stills each time before using. Owing to the fact that these small errors would probably be partly offset by the quantities of nitrogen that cling to the tubes each time, the errors would probably be less than indicated by the blanks. In almost all the work and in all the later work, however, the stills were steamed immediately before being used.

Summary

A. Fresh sewage ordinarily undergoes a rapid decomposition by which the total organic nitrogen is largely changed into ammonia nitrogen, the sum of the total organic nitrogen and ammonia nitrogen remaining approximately the same. This change can be almost entirely prevented by adding chloroform to the sewage and keeping it in a refrigerator.

B. If the ammonia nitrogen content does not exceed 25 parts per million, the ammonia nitrogen determination either by direct nesslerization or by distillation is accurate to ± 1 part per million.

C. The procedure most successfully employed for the determination of total organic nitrogen plus ammonia nitrogen was as follows: The sewage was filtered through absorbent cotton. Two hundred cc. of the filtrate was placed in a 800 cc. flask. To this was added 1 cc. of 10% copper sulfate solution and 10 cc. of concentrated sulfuric acid The liquid was digested without violent boiling for at least thirty minutes after it became colorless. To the hot liquid, cystals of potassium permanganate were added one at a time until a color persisted. After the liquid had cooled a few minutes, about 400 cc. of nitrogen-free water and 25 cc. of a 40% solution of sodium hydroxide were added. The flasks were connected immediately to the stills and 200 cc. was distilled

as quickly as possible into an Erlenmeyer flask containing a few cc. of nitrogen-free water and a quantity of N/50 sulfuric acid not quite sufficient to neutralize all the ammonia. The titration was completed with N/50 sulfuric acid in the presence of congo red as the indicator.

The author thinks that this method is accurate to ± 1 part per million in the absence of nitrates and nitrites. Lack of time did not permit a thorough comparison of the results obtained with and without the use of potassium permanganate. It may be said that at least one small crystal of the permanganate was usually decolorized by the hot liquid. This seemed to indicate that the sulfuric acid, copper sulfate mixture had not completely oxidized the organic matter and that the use of the permanganate was advisable.

D. A comparison of the results obtained by determining total organic nitrogen with and without first removing the ammonia nitrogen showed that very little if any nitrogen was lost by digesting the sample without first removing the ammonia nitrogen.

E. The investigation of the effect of the presence of nitrate and nitrite nitrogen on the determination of total organic nitrogen seems to point clearly to the conclusion that either nitrate or nitrite nitrogen cause loss of organic nitrogen. In samples containing 30 to 40 parts per million of total organic nitrogen plus ammonia nitrogen, the loss was roughly 4 to 5 parts per million when 10 parts per million of

nitrate or nitrite nitrogen was added. The method is, therefore, considered unreliable in the presence of considerable quantities of nitrates or nitrites.

F. The small quantities of nitrogen removed from dirty stills indicates that steaming out the stills is not necessary in sewage work.

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